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JOHNSON & JOHNSON MEDICAL LIMITED
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Patents ADP number *(if you know it)* — [illegible]

If the applicant is a corporate body, give the country/state of its incorporation — **United Kingdom**

4. Title of the invention — **STERILE THERAPEUTIC COMPOSITIONS**

5. Name of your agent *(if you have one)* — **Carpmaels & Ransford**

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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11. I/We request the grant of a patent on the basis of this application.

Signature Carpmaels & Ransford Date 7th December 1998

Carpmaels & Ransford

12. Name and daytime telephone number of person to contact in the United Kingdom **Dr. Anthony James** **0171 242 8692**

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STERILE THERAPEUTIC COMPOSITIONS

The present invention relates to sterile compositions comprising one or more therapeutic peptides. The present invention also relates to methods for the preparation of such compositions, and to methods for the preparation of sterile therapeutic peptides.

Natural and synthetic peptide compounds are assuming increasing importance as active agents in therapy. There is particular interest in the use of growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF) and transforming growth factors (TGF), especially for the treatment of wounds. Growth factors are becoming available in substantial quantities from recombinant DNA synthetic techniques, which raises a need for sterile formulations for administration of the growth factors.

A feature of peptide-based medicaments is that most peptides are not suitable for enteral administration, because they are broken down in the digestive tract. Therefore, most peptide medicaments must be administered parenterally, and in particular by topical or intravenous administration. For such methods of administration, it is absolutely essential that the peptide composition must be sterile.

A difficulty arises when attempting to sterilise compositions comprising therapeutic peptides. Conventional sterilisation methods, such as heating in an autoclave or gamma-irradiation, tend to decompose therapeutic peptides, especially if the sterilisation is carried out in the presence of water. In the past, peptide based medicaments have been formulated from sterile starting materials under aseptic conditions. Such aseptic manufacture is relatively expensive and difficult to perform. Peptide-containing solutions have also been sterilised by ultrafiltration, but this method is not suitable for compositions containing macromolecules, or for solid or semi-solid compositions.

EP-A-0238839 describes wound dressings comprising an absorbent cellulosic sheet impregnated with an enzyme such as trypsin. The wound dressings are prepared and dried under aseptic conditions, apparently without subsequent sterilisation.

EP-A-0312208 describes gel formulations containing polypeptide growth factors havi human mitogenic or angiogenic activity. The gel formulations are suitable for topical application to wounds, and include cellulose derivatives that are said to be capable of stabilising the polypeptide growth factors against loss of biological activity in aqueous solution. Methyl cellulose and hydroxyalkyl cellulose derivatives are preferred. The gels are sterilised before addition of the peptide growth factors, and addition of the peptide growth factors is then carried out under sterile conditions. It therefore appears that there is no sterilisation of the compositions after formulation. Similar compositions are described in EP-A-0267015.

EP-A-0308238 describes stable freeze-dried compositions comprising polypeptide growth factors. The freeze-drying stabilises the growth factors against loss of activity by hydrolysis in the water. The freeze-dried compositions include extenders such as water soluble or water swellable polymers, including cellulose derivatives. The specification discloses autoclave sterilisation of the starting materials other than growth factors, and sterile filtering of the growth factors, in order to produce a sterile freeze-dried product. The application apparently does not contemplate sterilising the final freeze-dried peptide composition.

WO95/02411 describes compositions comprising a polypeptide growth factor which provide long-term biochemical stability of the growth factor at 4°C. The growth factor is stabilized by providing the composition with a low pH in the range 2.8 to 3.8. However, sterilisation of compositions containing the growth factors is not described in this specification.

Oxidized cellulose is prepared by oxidation of some of the $-CH_2OH$ groups on cellulose to carboxylate groups by treatment with nitrogen dioxide or another oxidizing agent. Oxidised cellulose can be used to prepare bioresorbable and absorbent matrices capable of convenient application to tissue surfaces. Oxidised regenerated cellulose (ORC) fabrics are especially preferred, since they can be formed by oxidative treatment of regenerated cellulose fabrics, such as rayon-type fabrics. Two examples of such ORC fabrics are INTERCEED (Registered Trade Mark of Johnson & Johnson Medical, Inc.)

and SURGICEL (Registered Trade Mark of Johnson & Johnson Medical, Inc.). The INTERCEED fabric is used as an adhesion barrier in surgery. The SURGICEL fabric is used as a haemostat in surgery and in other applications. ORC offers important advantages over unmodified cellulose fabrics, in particular bioresorbability *in vivo*, and haemostatic properties.

EP-A-0437095 describes a process for preparing a neutralised oxidised cellulose product (nORC) by treatment of ORC with a solution of sodium acetate or the like. The neutralised product may be impregnated with acid-sensitive haemostatic agents, such as thrombin, to enhance its haemostatic properties, or with acid-sensitive adhesion preventive agents, such as tissue plasminogen activator (t-PA) to enhance its adhesion-prevention properties. In other embodiments, the cloth is impregnated with medicaments, such as growth factors.

Both ORC and n-ORC can be sterilised by gamma-irradiation, and EP-A-0437095 also teaches that n-ORC having heparin or heparin fragments complexed thereto can also be sterilised by gamma-irradiation. However, the reference does not describe sterilisation of ORC or n-ORC having a peptide associated therewith.

EP-A-0562864 describes composite wound dressing materials comprising a matrix of collagen sponge, a substructure of a second bioabsorbable polymer which may for example be dispersed fibers of ORC, and an active agent which may be a peptide. The active agent can be provided in the matrix, or in the substructure, or in both. The composite sponge materials can be packaged and sterilized. The materials described in this reference are inherently inhomogeneous. They comprise a substructure of fibers, films or flakes providing phasic release of active agents and, preferably, directional cellular ingrowth.

WO98/00180 describes the use of complexes of ORC with collagen for chronic wound healing. The specification notes that ORC forms stable complexes with cell growth factors.

The present invention is based on the surprising finding that peptide therapeutic agents, and in particular growth factors, can be stabilised against decomposition during sterilisation if the peptides are formulated with and effective amount of a biopolymer such as a structural protein or a polyanionic polysaccharide, in particular collagen and/or an oxidised cellulose, prior to sterilization.

In a first aspect, the present invention provides a sterile composition comprising a therapeutic peptide complexed to a biopolymer, said therapeutic peptide and biopolymer being dispersed in or on a pharmaceutically acceptable carrier.

The peptide may be any therapeutically active peptide, including hormones, antibodies, antibody fragments, natural and synthetic peptide antigens and antigen fragments. Preferably, the peptide is therapeutically active to promote wound healing. Suitable peptides include haemostatic agents such as thrombin, and anti-adhesion agents such as t-PA. Also included are therapeutic fragments of natural proteins, such as collagen fragments. The invention is equally suitable for both water-soluble and water-insoluble peptides. The peptide may have any molecular weight, for example in the range 1000-100000, preferably 4000-60000.

Preferably, the peptide comprises a growth factor having human mitogenic or angiogenic activity. More preferably, the growth factor is selected from the group consisting of fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α, TGF-β_1, TGF-β_2), nerve growth factors (NGF-α, NGF-ß), epidermal growth factor (EGF), insulin-like growth factor (IGF-I or IGF-II), or mixtures thereof.

The term "biopolymer" encompasses a broad range of natural and semi-synthetic biocompatible polymers including: structural proteins such as collagen, fibrin and laminin; modified structural proteins such as atelocollagen or pepsin-solubilized collagen; polysaccharides such as cellulose, starch, alginates, chitin, and the mucopolysaccharides; and modified polysaccharides such as oxidized celluloses, modified starches and chitosan. Preferably, the biopolymer is substantially insoluble in

water. Preferably, the biopolymer is selected from the group consisting of native and modified structural proteins, and polyanionic polysaccharides.

The term structural protein in this specification refers to collagen and other structural proteins such as fibrin or laminin. These are not therapeutically active peptides in the normal sense of the word. Complexes between such structural proteins and alginate are described in US-A-4614794. Complexes between such structural proteins and ORC are described in GB-A-2314842. Such complexes provide advantages in wound healing applications, especially for the treatment of chronic wounds.

Preferably, the structural protein consists essentially of native fibrous collagen.

The term "anionic polysaccharide" encompasses mucopolysaccharides such as heparin, hyaluronic acid, heparan sulphate, chondroitin sulphate and fragments and salts thereof. However, preferably, the anionic polysaccharide comprises a polycarboxylate such as an alginate or an oxidised cellulose. More preferably, the anionic polysaccharide comprises ORC or n-ORC.

Suitable ORC may be prepared by the method of US-A-3122479 or from commercially available INTERCEED® or SURGICEL® fabrics, or in powdered or fibrous form by milling such fabrics. Neutralized ORC may be prepared as described in EP-A-0437095. Lower molecular weight ORC fragments, including water-soluble fragments, may be prepared by alkali hydrolysis of ORC as described in WO98/00446.

The composition according to this aspect of the invention preferably contains a therapeutically effective amount of the peptide for the intended use. Typically, in the case of compositions for topical application to wounds, this would be in the range of 0.1 to 10,000 ppm by weight, more preferably 1 to 1,000 ppm by weight. Preferably, the weight ratio of the therapeutic peptide to the biopolymer in the composition is from $1:10^6$ to 1:10, more preferably from $1:10^5$ to 1:100, and most preferably from $1:10^4$ to 1:1000.

The therapeutic peptide is complexed to the biopolymer. That is to say, the molecules of the therapeutic peptides are associated with molecules of the biopolymer by covalent, ionic or Van der Waals bonding. Preferably, the therapeutic peptide is not covalently bonded to the biopolymer. The complex is typically formed by intimate mixing of the therapeutic peptide and the biopolymer at the molecular level, for example by co-dispersing the therapeutic peptide and the biopolymer in a solvent, followed by removal of the solvent.

Preferably, the complex of therapeutic peptide and biopolymer is in the form of solid or colloidal particles dispersed in the pharmaceutically acceptable carrier. Preferably, the particles are less than 50 μm in diameter, more preferably less than 10 μm, and most preferably less than 2 μm in diameter. The pharmaceutically acceptable carrier may be a solid surface or a solid matrix. However, preferably, the carrier is an aqueous liquid or gel in which the therapeutic peptide and biopolymer are dispersed. In certain preferred embodiments, the sterile composition according to the present invention is a viscous liquid or a gel suitable for topical administration to the human or animal body, especially to wounds.

In other preferred embodiments, the sterile composition according to the present invention is a liquid, for example a buffered saline, containing the therapeutic peptide and the biopolymer, and suitable for intravenous administration to the human or animal body.

In other preferred embodiments, the sterile composition of the present invention may comprise a solid pharmaceutical carrier, with the therapeutic peptide and the biopolymer coated onto a surface of the carrier. Preferably, the solid carrier is a woven or nonwoven fabric, a polymer film, or a web, suitable for application to a surface of a wound. Particularly preferred solid carriers are bioabsorbable solid carriers, especially woven or nonwoven ORC cloth. These embodiments may, for example, be manufactured by coating the therapeutic peptide onto a solid carrier of the biopolymer (or a solid carrier coated with the biopolymer), drying to form the peptide/biopolymer complex at the surface of the solid carrier, and sterilizing.

Preferably, the sterile compositions according to the present invention are sterile packaged. That is to say, they are preferably packaged in a sterile microorganism-impermeable container.

In a second aspect, the present invention provides a process for the preparation of a sterile therapeutic composition comprising the steps of: providing a complex of a therapeutic peptide with a biopolymer; sterilizing the complex; and dispersing the complex in or on a pharmaceutically acceptable carrier.

The preferred therapeutic peptide, biopolymer and pharmaceutically acceptable carrier are as herein before defined with respect to the first aspect of the invention.

Preferably, the complex is formed by dispersing the therapeutic peptide and the biopolymer in a solvent, followed by removing the solvent to leave the complex. Preferably, the solvent is an aqueous solvent, and more preferably it consists essentially of water. Preferably, the solvent is removed by freeze drying or solvent drying. This generally results in a highly porous biopolymer sponge having the therapeutic peptide contacted thereto. This material can then be milled for subsequent dispersal in the pharmaceutically acceptable carrier.

In other preferred methods, the biopolymer is in the form of a woven or nonwoven fabric, a polymer film, a web or a sponge, and is coated with the therapeutic peptide, preferably by dipping the biopolymer into a solution of the therapeutic peptide. The solvent is then removed by evaporation to leave the biopolymer carrier having the therapeutic peptide complex to the surface thereof.

The step of sterilizing the complex is preferably carried out on substantially anhydrous therapeutic peptide/biopolymer complex, more preferably containing less than 10% by weight of water. The sterilizing may be carried out by any of the conventional methods, including autoclaving, treatment of ethylene oxide. Preferably, the sterilizing is carried out by treatment with a gas plasma, for example in a STERAD machine. In other preferred embodiments the sterilizing is carried out with ionizing radiation such as ultraviolet light, electron beams or gamma irradiation. More preferably, the ionizing

radiation is gamma radiation, most preferably at a sterilizing dosage of at least 1 to 50 kGy of cobalt-60 radiation, preferably 20-30 kGy. Surprisingly, the irradiation of therapeutic peptides complexed to biopolymer does not substantially degrade the activity of the therapeutic peptide, despite the well known tendency of peptides to be degraded by gamma-irradiation. Without wishing to be bound by any theory, the surprising stability of the therapeutic peptides complex to biopolymers may be due to the biopolymer acting as a trap for free radicals produced by the radiation.

Preferably, the step of dispersing is carried out under aseptic conditions after the step of sterilizing. This is especially useful for sterilizing dry complexes of therapeutic peptides with the biopolymer, followed by dispersing the sterilized complex in a liquid, gel or semi-solid carrier under aseptic conditions to form the sterile therapeutic composition.

In a third aspect, the present invention provides a process for the preparation of a sterile therapeutic peptide comprising the steps of: providing a therapeutic peptide; forming a complex between the therapeutic peptide and a biopolymer; sterilizing the complex; followed by separating the therapeutic peptide from the complex under aseptic conditions to obtain the sterile therapeutic peptide.

This aspect of the invention again makes use of the remarkable ability of biopolymers to stabilise therapeutic peptides against decomposition under standard sterilizing conditions. The preferred therapeutic peptides, biopolymers and sterilization methods are as specified above for the first and second aspects of the present invention.

Preferably, the therapeutic peptide is separated from the sterilized complex simply by solvent extraction, preferably by an aqueous solvent. Thus, for example, a complex comprising a soluble therapeutic peptide with a water-insoluble biopolymer can be separated simply by dispersing the complex in water, followed by filtration or micro-filtration to separate the dissolved therapeutic peptide from the biopolymer. Other conventional aseptic separation methods, such as chromatography, may of course be used. The sterile therapeutic peptide may be further purified by chromatography or the

like, and may be concentrated by freeze drying. Preferably, the sterile therapeutic peptide is substantially pure.

To summarise, the present invention stabilises therapeutic peptides against decomposition under standard sterilisation conditions by forming a complex between the therapeutic peptide and a biopolymer. Preferably, the complex is anhydrous e.g. a freeze-dried complex. The resulting sterile complex can be formulated into conventional therapeutic products for topical or parenteral administration.

Figure 1 shows a chart of the activity of freeze-dried PDGF (comparative example) before and after sterilization with gamma-irradiation;

Figure 2 shows a chart of the activity of PDGF complexed with (i) collagen only (ii) ORC only, and (iii) a collagen/ORC composite, before and after sterilization with gamma-irradiation.

Example 1

A solid sponge of fibrous collagen complexed to platelet derived growth factor (PDGF) was prepared and sterilized as follows.

An aqueous slurry of acid-swollen native collagen fibers derived from the bovine corium was prepared as described in US-A-4614794 or US-A-4320201, the entire contents of which are expressly incorporated herein by reference. The solids content of the slurry was 1 wt.%. The slurry was acidified with 0.05M acetic acid to swell the collagen fibers. Then PDGF (Sigma Chemical Co.) was added to samples of the slurry at concentrations of 0 wt.% (control), 0.1 wt.%, 0-33 wt.% and 1 wt.% based on the weight of the collagen. The slurry was de-gassed. 30 g of each slurry sample was poured into a 100 cm^2 petri-dish, blast frozen, and then freeze-dried overnight.

The resulting collagen sponges were cut in half, and one half of each sponge was sterilized by irradiation with 2.5 kGy of ^{60}Co gamma-irradiation. The other half of each sponge was left non-sterile as a control.

Example 2

A solid sponge of fibrous collagen/ORC complexed to PDGF was prepared as described in Example 1, but with the addition of 80% by weight (based on the weight of the collagen) of milled SURGICEL® ORC fibers to the collagen slurry before addition of the PDGF. The resulting products are collagen/ORC sponges containing 0(comparative), 0.1, 0.33, and 1.0wt.% of PDGF based on the weight of collagen plus ORC.

Example 3

A solid matrix of ORC coated with PDGF was prepared as follows.

A SURGICEL® ORC cloth (2 cm^2) was dipped in a 1% aqueous solution of PDGF, dried at room temperature, and cut in half. One half was sterilized by gamma-irradiation as described in Example 1, and the other half was kept for comparison.

Example 4

A solid sponge of collagen/ORC complexed to aprotinin was prepared as decribed in Example 2, but with the addition of bovine lung aprotinin (Sigma Chemical Co.) in an amount of 0 wt.% (control) and 10 wt.% based on the weight of collagen + ORC.

Procedure 1

The effects of complexation and sterilization on the activity of PDGF were assessed as follows:-

A 24-hour releasate of each sample was prepared by incubating punch biopsies (6mm diameter) in 1ml Dulbecco's Modified Eagle's Medium (DMEM) and incubating at 37°C, 5% CO_2 in a humidified atmosphere (cell culture incubator - JJM Research 00067). Each sample eluant was tested in duplicate.

The biological activity of PDGF was assessed by proliferation using a methylene blue assay to quantify cell number. Briefly, AHDF (adult human dermal fibroblasts) were grown to 95% confluency, trypsinised, counted and re-plated in 10% FBS/DMEM at a cell density of 3 x 10^4 cells/ml in a 96 well microtitre plate (100µl/well -3000

cells/well). The cells were allowed to adhere and spread overnight at 37°C, 5% CO_2 (JJM Research 00067) in this medium. The medium was then removed, the cell monolayer washed with PBS, and the test sample or standard was added at 100µl/well. Each test sample was tested x8 and sterility was achieved by filtering through a syringe filter (0.2µm). Each sample was diluted depending on the estimated concentration of the PDGF incorporated; this was necessary to enable quantification of PDGF over a linear range. Human recombinant PDGF-BB was used as a standard, diluted in SF-DMEM and used over a concentration range 500ng/ml - 1ng/ml. The stimulant was incubated with the cells for a further 3 days at 37°C, 5% CO_2, after which the medium was removed and the cell monolayer fixed with FORMOL® saline. The cells were then stained with methylene blue, excess stain removed and the dye eluted from the cell monolayer with acidified ethanol. The solubilized dye was quantified at 630nm using a microtitre plate spectrophotometer and the data analysed using BIOLINX® software.

The total level of PDGF released from each sample type was determined by ELISA. A monoclonal capture antibody to PDGF-BB was used at 0.5µg/ml 0.025 M-$NaHCO_3$, 0.025M-Na_2CO_3, 0.025M-Na_2CO_3, pH 9.7 (100µl/well) and incubated overnight at 4°C. Remaining binding sites were blocked with 3% BSA/PBS for 1 hour 37°C after which the test sample or standard was added. Each sample was tested in triplicate and the standard curve ranged from 500ng/ml to 1ng/ml. Each sample was tested at various dilutions to allow an accurate estimation of concentration. The level of PDGF present in the test sample was quantified using a primary polyclonal antibody to PDGF (1/1000 dilution) and a secondary anti-rabbit IgG with a peroxidase conjugate (1/5000 dilution). The level of peroxidase was then quantified using a soluble substrate TMB initially forming a blue colour that turns yellow upon the addition of IM-sulphuric acid. The colour was monitored at 450nm spectrophotometrically.

Typical results are shown in Figure 2. It can be seen that the activity of the PDGF complexed to collagen, collagen/ORC or ORC alone is substantially unchanged by the sterilization. In contrast, the comparative study of pure freeze-dried PDGF shown in Figure 1 demonstrates the large decrease in PDGF activity when sterilization is performed on the pure PDGF growth factor. The control samples with no added PDGF showed no activity before or after sterilization.

Procedure 2

The effects of complexation and sterilization on the activity of aprotinin were assessed as follows.

3mm punch biopsies were taken in duplicate from collagen/ORC, collagen/ORC containing aprotinin (irradiated), and collagen/ORC containing aprotinin. Two punch biopsies from each sponge were added to 1 ml of DMEM in a sterile plastic container. The DMEM was removed after a number of timepoints ranging from zero - 48hrs incubation at 37°C. The releasates were stored at -20°C until required for the functional assay of aprotinin activity.

The effect of irradiation on the aprotinin functional activity was tested by measuring the ability of aprotinin to inhibit a sample of purified plasmin. Briefly, 10μls of releasate was added to a total assay volume of 100μls, containing 40μls of plasmin (40ng), 10μM plasmin fluorogenic substrate and Tris-HCl buffer (pH 8.1) containing 0.5% TRITON®. Positive controls were run in the absence of releasate.

In addition, a standard curve of plasmin inhibition over a variety of aprotinin concentrations from 0.5ng/ml - 10ng/ml was constructed and used as the basis for calculations.

The assay was monitored over 1hr at 37°C and the amount of fluorescence detected using a fluorogenic plate reader (emission 455nm, excitation 383nm).

The results showed that a substantial fraction of the aprotinin activity was preserved after gamma-irradiation, when the aprotinin was complexed to collagen/ORC.

Surprisingly, the results also showed that the aprotinin ws released more rapidly from the irradiated collagen/ORC matrix than from the non-irradiated collagen/ORC matrix.

Example 5

A sterile pharmaceutical gel for topical administration to promote wound healing was formulated as follows:-

Carboxymethyl Cellulose	2.4%
Hydroxyethyl Cellulose	0.3%
Sodium chloride	0.24%
Propylene glycol	20.2%
Collagen/ORC/PDGF 1 wt.%	2.0%
Water	balance

The Collagen/ORC/1 wt.% PDGF was prepared as described in Example 2, comminuted to a particle size of about 1-10μm, sterilized by gamma-irradiation, and then mixed with the other components under aseptic conditions to give a sterile aqueous gel suitable for application to wounds to promote wound healing.

Example 6

A sterile, substantially pure PDGF was prepared as follows.

The 1% PDGF/collagen sponge prepared in Example 1 was milled to particle size less than 10μm, then sterilized by gamma-irradiation. The sterile complex was treated with sterile saline (pH 8) for 1 hour at 35°C to extract the sterile PDGF, followed by filtration and freeze-drying under aseptic conditions to obtain sterile PDGF.

The above examples have been disclosed for the purpose of illustration only. Many other embodiments of the present invention falling within the scope of the accompanying drawings will be apparent to the skilled reader.

CLAIMS

1. A sterile composition comprising a therapeutic peptide complexed to a biopolymer, said therapeutic peptide and biopolymer being dispersed in or on a pharmaceutically acceptable carrier.

2. A sterile composition according to claim 1, wherein said peptide is selected from the group consisting of growth factors, haemostatic agents, antimicrobial agents, antibacterial agents, anti-adhesion agents and collagen fragments.

3. A sterile composition according to claim 2, wherein the peptide comprises a growth factor having human mitogenic or angiogenic activity.

4. A sterile composition according to claim 3, wherein the growth factor is selected from the group consisting of fibroblast growth factor (FGF), Platelet derived growth factor (PDGF), Transforming growth factors (TGF-α, TGF-β_1, TGF-β_2), Nerve growth factors (NGF-α, NGF-β), epidermal growth factor (EGF), insulin-like growth factors (IGF-I or IGF-II), and mixtures thereof.

5. A sterile composition according to any preceding claim, wherein said biopolymer is selected from the group consisting of structural proteins, polyanionic polysaccharides, and mixtures thereof.

6. A sterile composition according to claim 5, wherein said structural proteins are selected from the group consisting of native-collagen types, atelocollagen, pepsin-solubilized collagen, gelatin, fibronectin and laminin.

7. A sterile composition according to claim 6, wherein said structural proteins consist essentially of native fibrous collagen.

8. A sterile composition according to any one of claims 5 to 7, wherein said polyanionic polysaccharide is selected from the group consisting of oxidized cellulose

such as oxidized regenerated cellulose (ORC), neutralized ORC (nORC), hyaluronic acid, alginic acid, heparan, keratan, and salts and mixtures thereof.

9. A sterile composition according to claim 8, wherein said polyanionic polysaccharide is selected from the group consisting of ORC and nORC.

10. A sterile composition according to any preceding claim, wherein said biopolymer comprises a mixture of native fibrous collagen, and ORC.

11. A sterile composition according to any preceding claim, wherein the weight ratio of said therapeutic peptide to said biopolymer is from $1:10^6$ to 1:10.

12. A sterile composition according to claim 11, wherein said weight ratio os from 1 to 10^5 to 1:100.

13. A sterile composition according to claim 12, wherein said weight ratio is from 1 to 10^4 to 1:1000.

14. A sterile composition according to any preceding claim, wherein said therapeutic peptide and said biopolymer are in the form of solid or colloidal particles dispersed in said carrier.

15. A sterile composition according to claim 14, wherein said carrier is an aqueous liquid or gel.

16. A sterile composition according to any preceding claim, wherein said composition is a viscous liquid or gel for topical administration to the human or animal body.

17. A sterile composition according to any one of claims 1 to 15, wherein said composition is a liquid for intravenous administration to the human or animal body.

18. A sterile composition according to any one of claims 1 to 13, wherein said carrier is a solid, and said therapeutic peptide and biopolymer are coated onto a surface of said carrier.

19. A sterile composition according to claim 18, wherein said solid carrier is a woven or nonwoven fabric, a polymer film, or a web, suitable for application to a surface of a wound.

20. A sterile composition according to claim 18 or 19, wherein said solid carrier is bioabsorbable.

21. A sterile composition according to claim 20, wherein said solid carrier comprises a woven or nonwoven ORC cloth.

22. A sterile composition according to any preceding claim which is sterile packaged.

23. A process for the preparation of a sterile therapeutic composition comprising the steps of:

providing a complex of a therapeutic peptide with a biopolymer;
sterilizing said complex; and
dispersing said complex in or on a pharmaceutically acceptable carrier.

24. A process according to claim 23, wherein said step of providing comprises mixing said therapeutic peptide with said biopolymer in a solvent, followed by removing said solvent to leave said complex.

25. A process according to claim 24, wherein said solvent is an aqueous solvent.

26. A process according to claim 24 or 25, wherein said solvent is removed by freeze drying or solvent drying.

27. A process according to any one of claims 23 to 26 wherein said step of sterilizing is carried out with ionizing radiation or by treatment with a gas plasma.

28. A process according to claim 27, wherein said ionizing radiation is gamma radiation.

29. A process according to any one of claims 23 to 28, wherein said complex is substantially anhydrous during said step of sterilizing.

30. A process according to any one of claims 23 to 29, wherein said step of dispersing is carried out under aseptic conditions, after said step of sterilizing.

31. A process according to any one of claims 23 to 30 for the preparation of a sterile composition according to any one of claims 1 to 22.

32. A process for the preparation of a sterile therapeutic peptide comprising the steps of:

providing a therapeutic peptide;

forming a complex between the therapeutic peptide and a biopolymer;

sterilizing said complex; followed by

separating the therapeutic peptide from said complex under aseptic conditions to obtain said sterile therapeutic peptide.

33. A process according to claim 32, wherein said therapeutic peptide is separated from said complex by dispersing the complex in an aqueous solvent, followed by filtration.

34. A sterile composition substantially as hereinbefore described with reference to the examples.

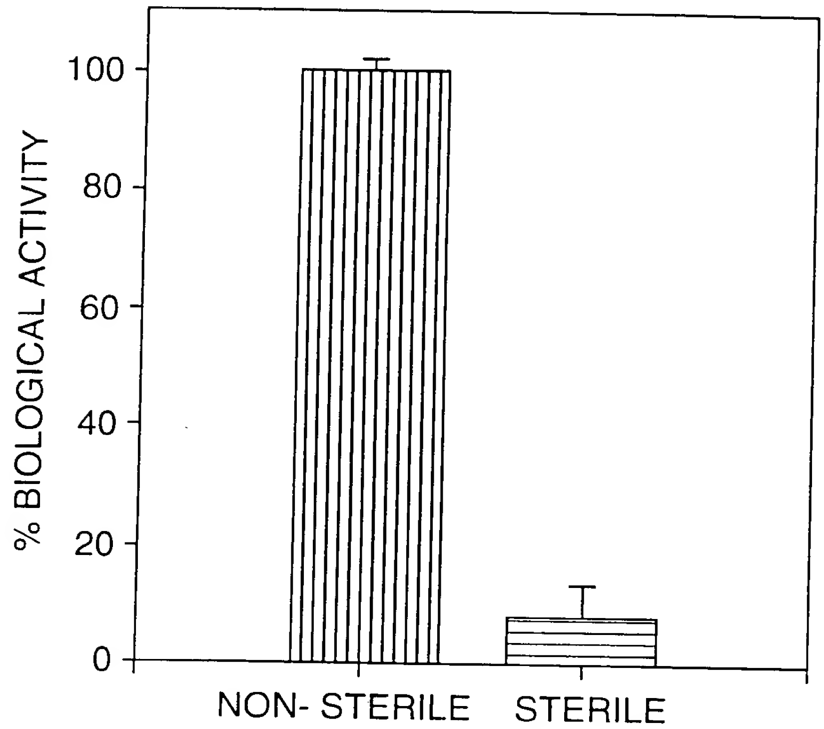
35. A process for the preparation of a sterile composition substantially as hereinbefore described with reference to the examples.

ABSTRACT

STERILE THERAPEUTIC COMPOSITIONS

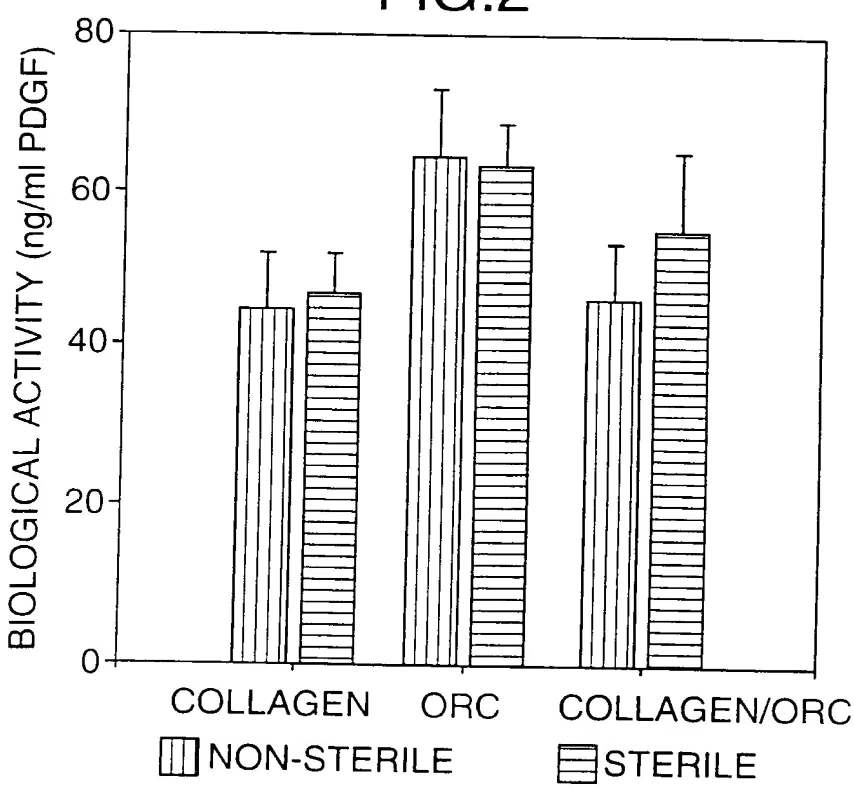
The invention provides sterile compositions comprising a therapeutic peptide complexed to a biopolymer, the complex being dispersed in a pharmaceutically acceptable carrier. The presence of a biopolymer such as collagen or oxidized regenerated cellulose surprisingly stabilizes therapeutic peptides against decomposition under conventional sterilization conditions, especially under gamma-irradiation. Processes for the preparation of the sterile compositions and processes for the preparation of sterile therapeutic peptides are also claimed.

FIG.1



% BIOLOGICAL ACTIVITY
100
80
60
40
20
0
NON- STERILE
STERILE

FIG.2



BIOLOGICAL ACTIVITY (ng/ml PDGF)
80
60
40
20
0
COLLAGEN
ORC
COLLAGEN/ORC
NON-STERILE
STERILE

PCT/GB 99/04094

6.12.99

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